

### **REMARKS**

Claim 18 has been amended to directly refer to gene identification in the step (g). Support for this amendment can be found throughout the specification and specifically, for example, page 3, last full paragraph.

Claim 25 has been amended following to the examiner's suggestion to distinctly point to the steps of embryonal DNA staining and analysis of the stained embryos. Support for this amendment can be found throughout the specification and specifically, for example, on pages 35-36.

Claim 29 has been amended following the examiner's suggestion to add steps describing the irradiation analysis. Support for this amendment can be found throughout the specification and specifically, for example, on page 16, first paragraph.

Accordingly, present amendments are supported by the specification and do not introduce new matter and therefore, their entry is respectfully requested.

Turning now to the specific rejections by the examiner.

Claims 18-29 were rejected under 35 USC § 112, 2<sup>nd</sup> par. as being indefinite.

To expedite prosecution, applicants have amended claims 18, 25 and 29 as shown above. Applicants wish to point out that one of the advantages of the method of the present invention is that one may identify a gene involved in cell proliferation before and without the actual positional cloning just on the basis of the phenotype. Therefore, the method as presented in claim 18 identifies a gene or mutation involved in carcinogenesis. Positional cloning of the gene may further be performed to isolate the gene but is not necessary for identification of the gene or its function.

Claim 25 has been amended to include DNA staining as a step of flow cytometry. DNA staining can be performed using a variety of known DNA stains as explained, for example, on page 12, second full paragraph and on pages 35-36.

Claim 29 has been amended following the examiner's suggestion to add steps describing

the irradiation analysis. Support for this amendment can be found throughout the specification and specifically, for example, on page 16, first paragraph.

Accordingly, applicants submit that the above described amendments to claims 18, 25, and 29 obviate the rejections of claims 18-29 under 35 USC § 112, 2<sup>nd</sup> par. and that the rejection should thus be withdrawn.

Claims 18-21, 23, and 29 were rejected under 35 USC § 103(a) as being obvious over Spitsbergen in view of **three different** secondary references including Driver, Cheng and Alexander.

Applicants strongly disagree. To establish a prima facie case of obviousness the examiner must consider the references as a whole and then show, without impermissible hindsight, that the references provides some motivation to one skilled in the art to combine them with each other and arrive at the claimed invention. The claims presently under examination provide a method of using mutant fish embryos in a first screen to identify cell proliferation mutants and then testing such identified cell proliferation mutant fish in a second screen to identify novel genes involved in carcinogenesis.

While the cited primary reference, Spitsbergen, describes exposing zebrafish to MNNG in a study to determine the carcinogenic effects of MNNG and concludes that MNNG causes neoplasia in zebrafish, nothing in Spitsbergen teaches or suggests that mutated fish can be used in to identify novel genes associated with carcinogenesis a screening method including a carcinogenesis screen. There is no discussion in Spitsbergen concerning identifying novel genes involved in carcinogenesis, nor does Spitsbergen teach or suggest a two-step screening method to study carcinogenesis as claimed by the applicants. All Spitsbergen teaches is that zebrafish embryos and fry show neoplastic growth after exposing them to MNNG carcinogen. Therefore, Spitsbergen provides no teaching of at least the steps a-e of claim 18.

Driver, the **first of the cited three** secondary references, teaches the use of zebrafish, in general, in the study of vertebrae development. Nowhere in Driver is there even a mention of use of the fish in identifying mutations using a cell proliferation marker, and then further

screening the fish in a carcinogenesis screen to identify whether the particular cell proliferation mutant is involved in carcinogenesis, i.e., steps d-g of claim 18.

Cheng, the **second of the cited three** secondary references, describes a comparison of two-generation, haploid and half-tetrad zebrafish screens with genetic mapping to identify genes, again, involved in development (see, e.g. last paragraph, col. 1, on page 528), not carcinogenesis. Moreover, even the examiner acknowledges that this article does not teach use of a marker to identify mutants in a fish embryo. Therefore, Cheng lacks teaching of at least the steps d-g of claim 18.

Alexander, the **last of the three** cited secondary references, is involved in identifying fish mutants with defects in cardiac development. Alexander uses nkx2.5 and gata-1 riboprobes in whole mount in situ hybridization in haploid zebrafish embryos to expedite screening for interesting mutations involved in cardiac development. Alexander does not teach or suggest the use of zebrafish to identify genes with cell proliferation defects and their consequent use in a carcinogenesis screen to identify genes involved in carcinogenesis. Therefore, Alexander does not teach at least the steps e-g of claim 18.

In light of the above, the **four cited references**, even in combination with Spitsbergen, do not teach all the steps of the claimed method. Moreover, all the secondary references describe use of a zebrafish for studying developmental processes, which has no bearing on identification of genes associated with carcinogenesis and thus not only do they lack explicit suggestion of the invention but are also related to a completely different field of research, development instead of carcinogenesis. Thus, in the light of the above it is evident that one skilled in the art would not have been motivated to combine all three of the cited secondary references with the primary reference, and further, even assuming, *arguendo*, that one were to combine them, the combination would not teach or suggest the invention as a whole.

Therefore, in the light of the above, applicants submit that the rejection of claims 18-21, 23, and 29 under 35 USC § 103(a) over Spitsbergen in view of Driver, Cheng and Alexander be withdrawn.

Claims 18-24, and 29 were rejected under 35 USC § 103(a) as being unpatentable over Spitsbergen, Driever, Cheng and Alexander in view of a **fifth reference**, Epstein.

Applicants strongly disagree. Spitsbergen, Driever, Cheng and Alexander were discussed above and the arguments are incorporated herein. Epstein merely teaches antisense oligonucleotides and their use as cell proliferation markers but does not in any way teach or suggest use of such probes in a zebrafish nor does Epstein teach a screen for novel carcinogenesis associated genes using zebrafish haploid screen followed by carcinogenesis assay. Therefore, Epstein does not overcome the deficiencies in the cited four other references. Further, the examiner has provided no indication that one of ordinary skill in the art, without knowledge of the claimed invention, would piece together the teachings of **five references** to result in the claimed invention. The only way to achieve such result is through impermissible hindsight obviousness. Consequently, applicants submit that the rejection of claims 18-24, and 29 under 35 USC § 103(a) over Spitsbergen, Driever, Cheng, and Alexander in view of Epstein be withdrawn.

Claims 18-21, 23, 25, and 29 were rejected under 35 USC § 103(a) as being unpatentable over Spitsbergen, Driever, Cheng and Alexander in view of Shyjan.

Applicants strongly disagree. Spitsbergen, Driever, Cheng and Alexander were discussed above and the arguments are incorporated herein. Shyjan teaches a method of using cell proliferation markers in the flowcytometric assay. There is, however, no teaching or suggestion that such method could be used in a double screening wherein mutant fish embryos are first screened for cell proliferation defects and such defective mutants be consequently screened in a carcinogenesis assay to show whether the cell proliferation defects are associated with carcinogenesis. Therefore, Shyjan does not overcome the deficiencies in the four other cited references. And again, the examiner has provided no indication that one of ordinary skill in the art, without knowledge of the claimed invention, would piece together the teachings of **five references** to result in the claimed invention. The only way to achieve such result is through impermissible hindsight obviousness. Consequently, applicants submit that the rejection of

claims 18-21, 23, 26, 27 and 29 under 35 USC § 103(a) over of Spitsbergen, Driever, Cheng, and Alexander in view of Shyjan be withdrawn.

Claims 18-21, 23, 26, 27 and 29 were rejected under 35 USC § 103(a) as being unpatentable over Spitsbergen, Driever, Cheng and Alexander in view of O'Reilly.

Applicants strongly disagree. Spitsbergen, Driever, Cheng and Alexander were discussed above and the arguments are incorporated herein. Like teachings of Epstein and Shyjan, wherein only specific marker or method, respectively, are taught, O'Reilly only teaches TUNEL as a method for identifying cell proliferation defects. Thus, O'Reilly does not overcome the deficiencies in the four other cited references. Like for the arguments discussed above, the examiner also here has provided no indication that one of ordinary skill in the art, without knowledge of the claimed invention, would piece together the teachings of **five references** to result in the claimed invention. The only way to achieve such result is through impermissible hindsight obviousness.

Therefore, applicants submit that the rejection of claims 18-21, 23, 26, 27 and 29 under 35 USC § 103(a) over Spitsbergen, Driever, Cheng and Alexander in view of O'Reilly be withdrawn.

Claims 18-21, 23, 28 and 29 were rejected under 35 USC § 103(a) as being unpatentable over Spitsbergen, Driever, Cheng and Alexander in view of Li.

Applicants again strongly disagree. Spitsbergen, Driever, Cheng and Alexander were discussed above and the arguments are incorporated herein. Li teaches that BrdU stain can be used as a diagnostic marker for tumors but this teaching does not overcome the deficiencies in the four other cited references. Once again, the examiner fails to provide any indication that one of ordinary skill in the art, without knowledge of the claimed invention, would piece together the teachings of **five references** to result in the claimed invention. The only way to achieve such result is through impermissible hindsight obviousness. Therefore, applicants submit that the rejection of claims 18-21, 23, 28 and 29 under 35 USC § 103(a) over Spitsbergen, Driever, Cheng and Alexander in view of Li be withdrawn.

Application No. 09/758,007  
Amendment dated May 19, 2003  
Reply to Office Action of January 17, 2003

In conclusion, one skilled in the art, without knowledge of the present invention and thus without the benefit of impermissible hindsight, would not have been motivated to combine the only cited **primary reference** discussing carcinogenic properties of MNNG on zebrafish **with the three or even four** additional secondary references discussing use of zebrafish in studying vertebrae development to arrive at the method of the claimed invention. Furthermore, even assuming, *arguendo*, that one were to combine the references, the combination would not teach or suggest all the steps of the present invention as discussed above.

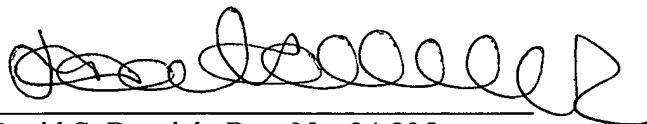
In view of the foregoing, applicant respectfully submit that all claims are in condition for allowance. Early and favorable action is requested.

In the event that any additional fees are required, the PTO is authorized to charge Nixon Peabody deposit account No. 50-0850.

Respectfully submitted,

Date:

May 19, 2003



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